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Accumulation of lead and zinc by plants colonizing a metal mining area in Central Iran

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Summary

The Irankouh area, located in Central Iran, is a vast mountainous region with mineralized soils and several active zinc and lead mining and smelting sites. In this study plants and soils from 5 different sites in this area were collected and analyzed for Zn and Pb. Analysis of soils from different sites showed the expected high concentrations of Zn and Pb - up to 23,000 and 18,000 µg g^{-1} for total, 30 and 20 µg g^{-1} for exchangeable, 1 and 0.6 µg g^{-1} for water-soluble fractions, respectively. Plants collected from these sites total 67 species from 66 genera and 29 families. Most of these are annual herbs found also on non-metalliferous soils in this region. The concentrations of Zn and Pb in the leaf dry matter of plants were variable, with up to 4800 µg g^{-1} for Zn and 740 µg g^{-1} Pb in *Matthiola chenopodiifolia* and *Pinus elderica*, respectively. A significant positive correlation was detected between the concentrations of Zn and Pb in plant dry matter and those in soils. The concentrations of Zn and Pb in the leaves of most species collected were significantly higher than for other plants from non-metalliferous soils. Some accumulator plants found in this area could have potential for soil clean-up by phytoextraction.

Introduction

Heavy metal contamination is a most serious form of environmental pollution and has received widespread attention owing to toxicity to a wide variety of organisms. High concentrations of heavy metals in the soils are due to both natural processes as a result of weathering of rock minerals, and human industrial activities such as mining and smelting. On metal contaminated soils, a range of plants which have evolved physiological mechanisms to tolerate heavy metal toxicity can survive and reproduce (BAKER, 1987). Some tolerant plants are restricted to these metalliferous soils, either absolutely or regionally, and are recognized as absolute or local metallophytes. Others, occurring on both contaminated and uncontaminated soils as tolerant and non-tolerant races in the same region, are recognized as pseudometallophytes (BAKER, 1987). The latter group can be divided into selective, indifferent and accidentals (BAKER and PROCTOR, 1990). Some endemic forms of tolerant plants have been described on metalliferous soils, as with the zinc floras in Western Europe (BROOKS, 1998). The tolerance of plants to heavy metals is achieved either by metal exclusion or metal accumulation.

Some plants on heavy metal-contaminated soils accumulate extraordinary concentrations of heavy metals into their above ground parts. According to BAKER and BROOKS (1989) 'hyperaccumulators' of Co, Cu, Cr, Ni or Pb are those containing more than 1000 µg g^{-1} metal on a dry weight basis; for Mn and Zn the critical concentration was set at 10000 µg g^{-1}. To date more than 450 metal hyperaccumulator species belonging to 45 families have been identified, of which about 18 and 5 species are zinc and lead hyperaccumulators, respectively, mostly from contaminated sites of Europe (SHIMWELL and LAURIE, 1972; REEVES and BROOKS, 1983; REEVES and BAKER, 2000). Many hyperaccumulators are endemic to metalliferous or metal contaminated soils and thus are absolute metallophytes. While Zn is an essential element for plants and is normally present at concentrations of 10-200 µg g^{-1}, Pb is neither essential nor beneficial in plant nutrition and is generally present at about 1-10 µg g^{-1} in plant tissues. Hyperaccumulation of Pb by plants is exceedingly rare, owing to the readiness with which it can be precipitated as the insoluble sulfate in the rhizosphere, hence minimizing potential uptake and transport to the aerial parts of the plants (BAKER and BROOKS, 1989).

There are about 8000 plant species in Iran, belonging to 150 families; about 1727 of these species are endemic to the country (JALILI and JAMZAD, 1999). Due to the shortage of rainfall in central Iran, most plant species in these areas are herbaceous. While there are numerous natural metalliferous and metal-contaminated soils in different parts of Iran, little information is available regarding their flora, concentration of metals in plants and any relationship between the concentrations of metals in plants and soils. The aims of this study were to identify the plant species growing on mineralized and contaminated soils in the Irankouh Zn and Pb mining area and to determine the concentrations of Zn and Pb in its soils and plants.

Materials and methods

Site description

The Irankouh mining area is located 20 km southwest of Isfahan in central Iran in a mountain area at 1670-2750 m above sea level, between 32° 28´ and 32° 37´ N, and 51° 31´ and 51° 37´ E, in an area about 25 km by 3 km. The climate is semi-dry and the average annual rainfall is about 130 mm, mostly in winter and to some extent in autumn and spring. The maximum and minimum temperatures in summer and winter in this area are about 45°C and -10°C, respectively. There are several Zn and Pb mining sites, mostly exploited as open mines; some have been active during the last 60 years. In some parts the surface soils naturally contain high concentrations of Zn and Pb. Owing to the mining activity, and the distribution of dust and spoil, surrounding soils in a vast area have been affected by Zn and Pb. Ore concentration using flotation methods, and smelting, are carried out close to the mining sites, where dust and contaminated water cover the surroundings. In the present study, for collection of soils and plants, three different sites near three open-air mines (site1, *Colahdarvazeh*; site 2, *Gooshfile*; and site 3, *Tapehsorkh*), one small spoil heap (site 4) and an area around the smelting operation (site 5) were selected.

Soil and plant sampling and analysis

During June 2003-July 2004, plants growing on the above sites were collected for identification, analysis and preservation of reference specimens. Soil samples were taken from 0-15 cm depth from the same areas as the plants. The soils were air-dried and sieved to <2 mm.

For the analysis of total Zn and Pb, subsamples of 4 g were ground to pass a -80 mesh sieve (<190 µm) and oven-dried at 70°C to constant weight. A further subsample of 0.5 g was transferred to a Kjeldahl digestion tube for extraction with 10 ml of a 3:1 HCl/HNO_3 mixture. Tubes were left at room temperature overnight and were then transferred to a heating block. Each was covered with an air condenser and the heating control adjusted so that the mixture refluxed gently at 80°C for 2 hours. After cooling, the digests were filtered through a moistened filter paper into 50 ml volumetric flasks. Flasks were made up to volume with distilled water. Analysis for Zn and Pb was performed by atomic absorption spectrophotometry (AAS, Phillips model PU 9100).

For the analysis of exchangeable elements, 20 g of air-dry soil which had been sieved to <2 mm was placed in a 100 ml screw-cap polythene bottle, 50 ml of 1M NH_4NO_3 solution added, and the suspension was shaken for 2 hours at 20°C in an end-over-end shaker. After shaking, the soil suspension was left to stand for 5 min, and then filtered into a clean bottle. The filtrate was then acidified to 0.2% HNO_3 for analysis of the above elements by AAS. For the analysis of water-soluble elements, a 3-inch pot was filled with soil which had been sieved to <2 mm and watered with distilled water via its saucer. The saucer was refilled as required over 4 days to allow the sample to approach filled capacity. A conical flask with side-arm extension and a Büchner funnel with a 9 cm filter paper were placed on a top pan balance. The flask was connected to a vacuum line and wet soil from the pot was added gradually until the funnel was full. After 10 min, sufficient H_2O-extractable had been drawn into the flask and the apparatus was disconnected. The apparatus including the collected solution and the Büchner funnel was reweighed. The H_2O-extractable was decanted into an AAS tube and acidified with concentrated HCl to produce a final concentration of 4% HCl. The above elements were measured by AAS. The soil in the Büchner funnel was spread out and air-dried for a week and then reweighed. The concentration of Zn and Pb in H_2O-extractable could then be related to the soil in terms of w/w (dry weight) by calculating and compensating for water content. The pH of the soil was determined using a glass electrode after 10 g of soil had been stirred well in 30 ml distilled water in a beaker and allowed to stand for about 30 min.

For the analysis of plants for Zn and Pb, leaf materials were washed well with double distilled water and dried at 70°C for 48 h. Then about 0.5 g dry leaf sample was weighed into 25 ml beakers and ashed in a muffle furnace for 14 h at 480°C. The ash was taken up in 5 ml 10% HNO_3 and the digest finally made up to 10 ml in 10% HNO_3. The solutions were analyzed for Zn and Pb by AAS.

Results

The soils of the Irankouh mining area are of near neutral pH, except site 5 (pH 6), and are highly enriched with Zn and Pb (Tab. 1). The total concentrations are as high as 23500 µg g^{-1} Zn in site 1 and 18000 µg g^{-1} Pb in site 5 (Tab. 1). For exchangeable metals, the highest concentrations of Zn are 25-30 µg g^{-1} (sites 5 and 1), and the highest Pb concentrations are 17-21 µg g^{-1} (sites 1 and 5). For H_2O-extractable metals, the concentrations are low, only up to 1 µg g^{-1} Zn and 0.6 µg g^{-1} Pb (both at site 5) (Tab. 1).

In our study, 67 species of vascular plants were collected from different sites of the Irankouh mining areas (Tab. 2). They belong to 66 genera and 29 families. Major families represented are: Asteraceae (10), Brassicaceae (9), Poaceae (6), Apiaceae (5), Lamiaceae (5) and Chenopodiaceae (4). Most plants are herbaceous annuals, biennials or perennials. There are only 6 shrubs and 3 man-planted tree species: *Pinus eldarica*, *Platanus orientalis* and *Elaeagnus angustifolia*. These trees have been planted around sites 5 and 2.

The concentrations of Zn and Pb in the leaves of collected plants are shown in Tab. 2. The Zn concentrations ranged from 35 µg g^{-1} in *Peganum harmala* (site 2) to 4800 µg g^{-1} in *Matthiola chenopodiifolia* (site 1). In addition *Ebenus stellata* (site 1), *Heliotropium lasiocarpum* (site 5), *Diptychocarpus strictus* (site 1), *Phragmites australis* (site 5), *Kochis* sp. (site 4), *Descurainia sophia* (site 5), *Scorzonera tortuosissima* (1), *Tamarix ramosissima* (site 5) and *Capparis spinosa* (site 5) contained Zn at 2000-3000 µg g^{-1}.

Concentrations of Zn in 23 species were more than 1000 µg g^{-1}, belonging to site 1 (10 species), site 5 (9 species), site 3 (2), site 2 (1 species) and site 4 (1 species). The range of Pb concentrations was between 8 µg g^{-1} in *Acantholimon aspadanum* (site 2) and 740 in *Pinus eldarica* (site 5). *Elaeagnus angustifolia* (site 5), *Scorzonera tortuosissima* (site 5) and *Stachys inflata* (site 3) contained up to 600-700 µg g^{-1} Pb. Concentrations of Pb in 15 species were more than 300 µg g^{-1}, belonging to sites 1, 5 and 3 with 9, 5 and 1 species, respectively. There were 9 species with both >1000 µg g^{-1} Zn and 300 µg g^{-1} Pb in their leaves; among these, *Scorzonera tortuosissima* and *Ebenus stellata* contained 2400 and 2900 µg g^{-1} Zn and 650 and 500 µg g^{-1} Pb, respectively. There was no significant positive correlation between the concentrations of Zn and Pb in these 9 species ($r=0.05$, $p>0.05$).

There was a significant positive correlation between the concentrations of Zn in 20 species with more than 1000 µg g^{-1} Zn and the

Tab. 1: Means (n=5) and ranges (below) of total (T), exchangeable (E) and H_2O-extractable (H_2O-E) Zn and Pb concentrations, and soil pH for the study sites.

Site No.	Zn (µg g^{-1})			Pb (µg g^{-1})			pH
	T	E	H_2O-E	T	E	H_2O-E	
1	16350	18.3	0.32	3700	13.5	0.35	7.0
	10000-23500	10-30	0.30-0.35	1200-6400	10-17	0.30-0.40	6.9-7.1
2	5850	2.0	0.29	2150	1.35	0.25	6.9
	4400-7700	1.5-2.5	0.27-0.31	1900-2400	1.2-1.5	0.20-0.30	6.8-7.0
3	5150	11.0	0.35	4200	2.25	0.36	7.2
	3000-8500	10-12	0.30-0.40	3000-5700	2.0-2.35	0.30-0.40	7.1-7.3
4	11000	19.5	0.41	2900	3.4	0.31	7.4
	6500-18500	18-21	0.40-0.42	1400-4400	3.0-4.0	0.25-0.35	7.35-7.46
5	5700	23.5	0.90	14800	20	0.57	6.0
	5200-6250	22-25	0.80-1.00	12000-18000	18-21	0.55-0.60	5.9-6.2

Tab. 2: Concentrations of Zn and Pb in leaf dry matter (µg g^{-1}) of plants from sites in the Irankouh mining area.
Key: AH: annual herb; BH: biennial herb; PH: perennial herb; S: shrub; T: tree
†Single specimens, or range of values where 2-4 specimens were analyzed.

Species	Plant form	Site	Zn†	Pb†
Apiaceae				
Eryngium billardieri F. Delaroche	AH	2	80	150
Ferula sp.	AH	1	135	45
Psammogeton canescens (DC.) Vatke	AH	1	420	65
Pycnocycla spinosa Decne. ex Boiss.	AH	1,2	330-515	175-205
Zosima radians Boiss. & Hohen.	AH	1	800	115
Asclepiadaceae				
Cynanchum acutum L.	PH	1	1135	70
Asteraceae				
Acroptilon repens (L.) DC.	AH	5	920	240
Anthemis gayana Boiss.	AH	3	365	80
Artemisia sieberi Besser	AH	3	220	60
Centaurea gaubae (Bornm.) Wagenitz	AH	3	180	58
Centaurea ispahanica Boiss.	AH	1	730	380
Koelpinia tenuissima Pavl. & Lipsch.	AH	1	1360	360
Pulicaria gnaphaloides (Vent.) Boiss.	PH	2	260-305	40-105
Scorzonera tortuosissima Boiss.	AH	1,5	750-2400	270-650
Senecio glaucus L.	AH	1	1030	370
Zoegea purpurea Fresen.	AH	2	120	45
Boraginaceae				
Heliotropium lasiocarpum Fisch. & C. A. Mey.	AH	5	1120-2800	220-250
Lappula sp.	AH	3	355	70
Brassicaceae				
Cardaria draba (L.) Desv.	PH	1,4	600-675	50-90
Descurainia sophia (L.) Webb ex Prantl	AH	5	2125	450
Diptychocarpus strictus (Fisch.) Trautv.	AH	1	455-2670	185-335
Erysimum crassicaule (Boiss.) Boiss.	BH	5	1800	150
Isatis cappadocica Desv.	PH	1	465	65
Malcolmia africana (L.) R. Br.	PH	1,4	490-1050	20-115
Matthiola chenopodiifolia Fisch. & C.A. Mey.	PH	1	500-4800	160-340
Pseudocamelina sp.	BH	1	520-1280	100-180
Sisymbrium septulatum DC.	AH	5	1725	310
Capparaceae				
Capparis spinosa L.	PH	1,5	860-2460	45-230
Cleome foliolosa DC.	AH	2,3	340-535	50
Chenopodiaceae				
Anabasis setifera Moq.	PH	4	570	160
Girgensohnia oppositiflora (Pall.) Fenzl	AH	1	665	25
Kochis sp.	AH	4	2780	225
Salsola kali L.	PH	3,4	430-1390	25-86
Dipsacaceae				
Scabiosa olivieri Coult.	AH	1	580	350
Elaeagnaceae				
Elaeagnus angustifolia L.	T	5	520	660
Ephedraceae				
Ephedra strobilacea Bunge ex A. Lehm.	S	1,2	45-85	10-30
Euphorbiaceae				
Euphorbia striatella L.	PH	1	800	100
Fabaceae				
Astragalus sp.	PH	1	690	315
Alhagi persarum Boiss. & Buhse	PH	1	1800	180
Ebenus stellata Boiss.	S	1	2910	500
Geraniaceae				
Erodium oxyrrhynchum M. Bieb.	AH	1	400	300

Lamiaceae				
Hymenocrater incanus Bunge	PH	2	350	52
Lallemantia royleana Fisch. & C.A. Mey.	AH	3	430	60
Salvia sp.	AH	3	140	40
Stachys inflata Benth.	AH	1,2,3	295-1635	154-640
Ziziphora tenuior L.	AH	3,4	265-430	115-155
Malvaceae				
Alcea aucheri (Boiss.) Alef.	PH	2	160	30
Moraceae				
Ficus johannis Boiss.	S	1	1175	190
Papaveraceae				
Roemeria hybrida (L.) DC.	AH	3	430	40
Pinaceae				
Pinus eldarica Medw.	T	5	600	740
Platanaceae				
Platanus orientalis L.	T	2	125	50
Plumbaginaceae				
Acantholimon aspadanum Bunge	AH	2	50	8
Poaceae				
Bromus tectorum L.	AH	3	430	60
Eremopyrum orientale (L.) Jaub. & Spach	AH	3	1000	25
Pennisetum orientalis L. C. Rich.	PH	1	435	85
Phragmites australis (Cav.) Trin. ex Steud.	PH	1,5	950-2120	70-120
Poa sinaica Steud.	PH	1,3	105-350	30-50
Stipa barbata Desf.	PH	1	210	50
Polygonaceae				
Pteropyrum aucheri Jaub. & Spach	S	1	235	130
Resedaceae				
Reseda lutea L.	AH	4,5	725-1050	60-215
Rosaceae				
Amygdalus lycioides Spach	S	3	100	20
Scrophulariaceae				
Linaria michauxii Chav.	AH	1	400	50
Scrophularia striata Boiss.	PH	3	285	40
Solanaceae				
Solanum nigrum L.	AH	2	160	50
Tamaricaceae				
Tamarix ramosissima Ledeb.	S	5	2600	97
Urticaceae				
Parietaria judaica L.	PH	3	100	50
Zygophyllaceae				
Peganum harmala L.	PH	2	35	20

concentrations of total ($r=0.45$, $p<0.05$), exchangeable ($r=0.48$, $p<0.05$) and H_2O-extractable ($r=0.45$, $p<0.05$) Zn in the soil. Also, a significant positive correlation exists between the concentrations of Pb in 14 species with more than 300 µg g^{-1} Pb and the concentrations of total ($r=0.72$, $p<0.05$), exchangeable ($r=0.58$, $p<0.02$) and H_2O-extractable ($r=0.56$, $p<0.05$) Pb in the soil.

Discussion

The soils of all sites studied contain predictably high concentrations of Pb and Zn. This is due to natural mineralization in the parent rocks and to contamination by mining and smelting during the last 60 years. The concentrations of Zn and Pb in sites 1-3, shown in Tab. 1, are generally typical of mineralized soils in this area, although contaminated dusts arising from mining activity to some extent may have added to these elevated concentrations. High concentrations of these metals in site 4 are due to the spoil heap which has been leveled and left unchanged for nearly 20 years, whereas the high concentrations in site 5 are mainly from the smelting operation. There is considerable variation in concentration both within and between sites pointing to the heterogeneous nature of mineral and mine wastes (Ghaderian, 1998; Wenzel and Jockwer, 1999; Dahmani-Müller et al., 2000). Concentrations of Zn in sites 1-4 are higher than those of Pb, mainly because the Irankouh mining area is more enriched with Zn minerals, and Pb is the secondary metal. Much of the metal is insoluble and not immediately available for plants and is therefore not directly toxic. However, heavy metals in the H_2O-extractable and exchangeable forms may be directly accessible to organisms in the soil (LORENZ et al., 1997; POLLARD et al., 2002). Thus, the high concentrations of Zn and Pb in the H_2O-extractable and exchangeable fractions at some sites suggest extreme toxicity of the soil. According to PRÜESS (1994) the limits of NH_4NO_3-exchangeable Zn and Pb in soil are 5 and 0.3 μg g^{-1}, respectively; above these concentrations, the assessment of risk and remediation needs is required. These concentrations are exceeded in all the soils in this study, except for exchangeable Zn in site 2. The highest concentrations of mean Zn and Pb (exchangeable and H_2O-extractable) were in site 5, where minerals were processed and smelted. The pH here is 6 and due to the operations here the soils have more moisture. Generally, solubility and availability of metals in soils depend on many factors, including total metal content, pH and moisture regime (ALLOWAY, 1995).

Most plants collected in this area were annual or perennial herbs; this dominant vegetation form is due to the climate of the area, with low annual rainfall typical of Central Iran. Among the plants collected, 15 species are endemic to Iran (RECHINGER, 1963-1997), but not exclusively endemic to these metalliferous areas. As these plants naturally grow on these metal substrata, they can be categorized as pseudometallophytes (BAKER, 1987; POLLARD et al., 2002). Whether these plants are physiological races of metal tolerant species should be clear in further investigations.

According to the definition of BAKER and BROOKS (1989), the criteria for hyperaccumulation for Zn and Pb are >10000 and 1000 μg g^{-1}, respectively. On this basis, none of the collected species in the Irankouh mining area was a hyperaccumulator of either metal. The highest concentration of Zn (4800 μg g^{-1}) was recorded in leaves of *Matthiola chenopodiifolia (*Brassicaceae), collected from different parts of site 1. The highest Pb concentrations (above 500 μg g^{-1}*)* were found in planted trees of *Pinus eldarica* (740 μg g^{-1}) and *Elaeagnus angustifolia* (660 μg g^{-1}), as well as in naturally growing plants of *Scorzonera tortuosissima* (650 μg g^{-1}), *Stachys inflata* (640 μg g^{-1}) and *Ebenus stellata* (500 μg g^{-1}). To date 18 and 5 hyperaccumulators of Zn and Pb, respectively, have been reported (REEVES and BAKER, 2000), mostly from Europe, but Zn and Pb hyperaccumulation does not occur in most plants from Pb and Zn contaminated soils (SHIMWELL and LAURIE, 1972; REEVES, 1988; WENZEL and JOCKWER, 1999; REEVES et al., 2001). In contrast, Ni hyperaccumulators are rather more abundant in temperate and tropical ultramafic areas (REEVES, 1992; REEVES et al., 1999). YANG et al. (2002) believed that the criterion of 10000 μg g^{-1} Zn is too high for defining a Zn hyperaccumulator plant, and suggested 3000 μg g^{-1} leaf dry wt instead. Thus, the Zn accumulation in *M. chenopodiifolia* is notable and this plant seems to have potential for phytoremediation in some metal contaminated areas. Although there was no Pb hyperaccumulator at Irankouh, the existence here of 3 naturally occurring plants with >500 μg g^{-1} Pb is notable.

The significant positive correlations between the mean concentrations of Zn and Pb in the soil (as total, exchangeable or H_2O-extractable) and the mean concentrations of these metals in dry leaves of plants which accumulate >1000 and 300 μg g^{-1}, respectively, could indicate a cause-and-effect relationship. Such correlations can indicate a metal-accumulation tolerance strategy. BAKER et al. (1994), however, did not find such a correlation between indices of metal tolerance in different populations of *Thlaspi caerulescens* and concentrations of the metals in the soils. As some soils of the Irankouh area naturally contain high concentrations of Zn and Pb, most plants growing on these substrata should be adapted to these metal stress conditions. More work should be done to determine the correlations of the Zn and Pb concentrations of each accumulator plant with its own substratum.

However, it is possible in areas adjacent to mines and smelters, in particular, that part of the measured heavy metals may be from external deposition not removed in sample washing. It is almost impossible to know how much might have been externally deposited on leaf surfaces. One consequence, unfortunately, is that because the contaminant is essentially soil material, it will generate strong positive correlation between soil metal and that *apparently* present in the plant. This is not a great problem when plant analysis is used as a biogeochemical prospecting method, but can introduce uncertainties into discussions of the physiology of heavy metal uptake into plants from the soil via the soil solution and the root system.

Conclusion

Plants on Zn and Pb enriched soils of the Irankouh area were collected and identified, and the concentrations of these metals in the soils and plants were determined. None of the 67 collected species were recognized as true endemics on these substrata: all were pseudometallophytes. Total Zn and Pb concentrations in the soils were high, in the range 3000-23500 μg g^{-1} Zn and 1200-18000 μg g^{-1} Pb. The bioavailable concentrations of these metals as NH_4NO_3-exchangeable and H_2O-extractable were high enough to produce toxicity to nontolerant plants. The concentrations of Zn and Pb in the plant leaves indicated that most contain elevated amounts of these metals. The concentrations of Zn were up to 4800 and 2800 μg g^{-1} in *Matthiola chenopodiifolia* and *Heliotropium lasiocarpum*, respectively. The highest Pb concentrations were in planted trees of *Pinus eldarica* (740 μg g^{-1}) and *Elaeagnus angustifolia* (660 μg g^{-1}) and in the naturally growing species *Scorzonera tortuosissima* (650 μg g^{-1}) and *Stachys inflata* (640 μg g^{-1}). Despite high concentrations of bioavailable metals in the rhizosphere, no species showed hyperaccumulation of Zn or Pb.

There were significant positive correlations between the concentrations of Zn or Pb in the soils and in the leaves of accumulator plants. This suggests the adaptation of some metal tolerant plants to the toxic concentrations of heavy metals in the soils by the uptake mechanism. These accumulator plants may be valuable in phytoextraction of metal-contaminated soils in some parts of Central Iran.

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